Elucidation of Polymorph Mixtures Using Solid-State ^{13}C CP/MAS NMR Spectroscopy and Direct Exponential Curve Resolution Algorithm

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Abstract: Polymorphic behavior has a broad impact on chemical technology influencing the solid-state properties of many materials from pigments to pharmaceuticals. The simultaneous presence of two or more polymorphs may introduce ambiguities in the characterization of materials using spectroscopy, diffraction, or scattering techniques. In this paper we present a solid-state ^{13}C NMR method, together with the recently published direct exponential curve resolution algorithm, allows the elucidation of component spectra from the total spectrum of a mixture of polymorphs, previously characterized by X-ray diffraction.

Introduction

Many organic solids can crystallize in more than one crystal structure, a behavior known as polymorphism. Polymorphs differ in their solubility, color, and many other physical, chemical, and mechanical properties that are key to their end use in the pharmaceutical or materials sciences. The ability to detect the presence of different polymorphs in a sample, assay their relative amount, and ultimately obtain the desired form in very high yield are key issues in chemical technology.

Early high-resolution solid-state NMR experiments had already shown that ^{13}C chemical shifts are influenced by crystal packing.[1] Since then, several groups have reported that the differences in crystal packing lead to different sets of chemical shifts in the ^{13}C cross-polarization/magic angle spinning (CP/MAS) NMR spectra of the same compound in its different polymorphic forms.[2] The chemical shift data have been interpreted qualitatively in terms of differences in conformation among the various forms and might be made quantitative based on theoretical calculations of the full chemical shift tensors.[2d] Thus, ^{13}C solid-state NMR spectroscopy offers the promise of a sensitive probe for the characterization of polymorphic behavior. The ability to determine the simultaneous presence of multiple polymorphs in the same sample, and distinguish their NMR spectra, is an important part of such characterization.

Previous work from our laboratory[3a] has described a mathematical method for self-modeling mixture analysis, now known as direct exponential curve resolution algorithm and commonly referred to as DECRA. This method was applied successfully to the resolution of the NMR spectra of mixtures obtained by the pulsed field gradient spin−echo (PGSE) experiment[3] and to the resolution of MRI images.[4] An important feature of the algorithm that makes it paricularly suitable for the NMR characterization of complex mixtures is its insensitivity to even severe spectral overlap. In the PGSE NMR case the individual components of the mixture are labeled by their self-diffusion coefficients, D, while the components of a composite MRI image are differentiated by their transverse relaxation time, T_2, or longitudinal relaxation time, T_1. The key to the analysis is the exponential dependence of the signal from the individual components on the physical property being measured (i.e., D, T_2, or T_1). The results have shown that under optimal conditions DECRA can quantitatively separate the spectra of components whose exponential constants differ by as little as 20%. Instabilities in the performance of the instrument that lead to variations in baseline and line shape within a data set can, however, degrade the performance of the algorithm.

Protons in a homogeneous crystalline solid sample display a single spin−lattice relaxation time, T_1, regardless of chemical differences, because strong dipolar couplings among them lead to efficient spin diffusion, which in turn maintains a uniform spin temperature. Thus, proton spin−lattice relaxation times can be, and often are, phase-specific. It is well-known[5] that these differences can be exploited to selectively excite the ^{13}C CP/MAS NMR spectra of individual phases in a grossly heterogeneous solid. This is accomplished by inserting an inversion−recovery sequence on the protons prior to the cross-polarization step. This sequence consists of a $180°−\tau−90°$ pulse pair. For a judicious choice of the time interval τ, the proton magnetization in one of the phases goes through null, and therefore only

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the ^{13}C signal from the other is excited by CP and observed. For these techniques to be successful as currently applied, however, the differences in proton T_1's among the various phases need to be several-fold, a situation not always encountered in crystalline solids. Even if the proton T_1 differences are large, quantifying the relative amounts of the various phases can be rather cumbersome. Analysis of the experimental data by the DECRA approach holds out the promise of addressing both issues.

In the present paper we report on using DECRA in combination with a modified inversion–recovery pulse sequence to accurately decompose the ^{13}C CP/MAS spectrum of a mixture of polymorphs into its constituent subspectra. We illustrate this approach using compound **1**, which is known to exhibit complex polymorphic behavior. Two of the polymorphs have been isolated in pure form (henceforth referred to as type I and type III), and their structures have been determined by X-ray crystallography.

1
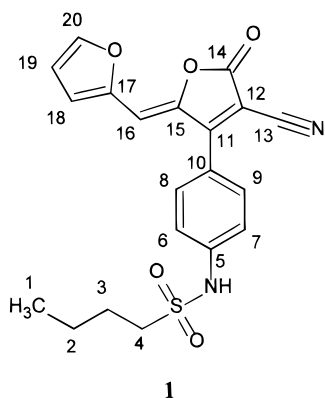
We find that the two forms have distinct ^{13}C NMR spectra and proton T_1 values. More significantly, we show that because of the phase-specific differences in proton T_1's, the individual ^{13}C subspectra of type I and type III can be unambiguously extracted from the spectrum of a 1:1 mixture of the two forms by applying the DECRA approach.

Experimental Section

The preparation of the yellow form, or type I polymorph, of **1** has been described previously.[6] The occurrence of the orange form, or type III, was observed unexpectedly as an impurity of a different color in a production batch of type I upon milling. A 2-propanol slurry of that batch containing 0.61 g of type I was held at 25 °C for 40 h with constant agitation. The resulting solids were filtered and submitted for X-ray powder diffraction analysis. The X-ray pattern obtained in this experiment was found to be different from that of type I. In a subsequent experiment, 2.0 g of type I was heated to 60 °C in 50 mL of 2-propanol. The resulting slurry, still at 60 °C, was seeded with 0.09 g of the type III obtained from the above, stirred for a further 15 min, and then allowed to cool to room temperature overnight with continued stirring. After 24 h, solids were collected by filtration and characterized by powder X-ray diffraction and differential scanning calorimetry. Both techniques confirmed the presence of type III. Further, there was no indication that any type I had survived the treatment. Both materials display identical ^{13}C spectra in solution.

The solid-state NMR spectra were obtained on a Chemagnetics CMX 300 spectrometer using Spinsight 2.5 software. The ^{13}C solid-state spectra of the two polymorphs and their 1:1 mixture were obtained using the standard CP/MAS experiment. Experimental details are given in the figure captions. A modified inversion–recovery experiment first
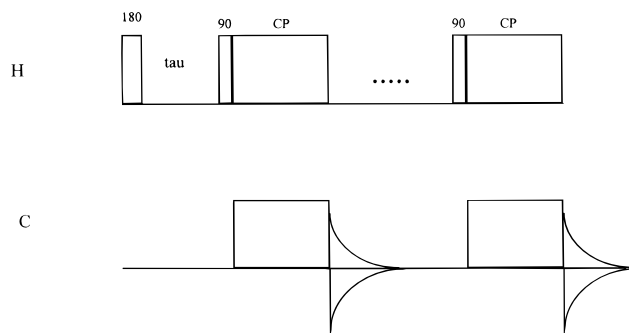
Figure 1. Pulse sequence used to collect data for DECRA analysis. Each spectrum in the data set is the signal-averaged difference between the first and second free induction decay for a given τ value. Typically 10 τ values are used.

suggested by Freeman and Hill[7] was combined with cross-polarization to obtain the spectral data set from the 1:1 mixture to which DECRA was applied. The pulse sequence is shown in Figure 1. The first 180° pulse inverts the proton magnetization into the *z*-axis of the rotating frame. During the variable interval τ the longitudinal proton magnetization partially relaxes toward equilibrium. It is then converted to transverse magnetization by the 90° pulse and subsequently transferred to the ^{13}C spins during the CP step. This sequence is immediately followed by a regular cross-polarization sequence, and the difference of the two signals is stored and accumulated as suggested by Freeman and Hill. This modification ensures that the data decay exponentially to zero for long τ values. Ten ^{13}C CP/MAS spectra were collected for values of τ ranging from 0.2 to 3.8 s in increments of 0.4 s. Each spectrum contained 2048 real data points.

The goal of the DECRA analysis is threefold: (a) determine the number of components in a mixture of solids, (b) determine their proton T_1 values, and (c) resolve the mixture into its pure spectral components. DECRA employs the generalized rank annihilation method (GRAM),[8] which requires two proportional data sets for the analysis. We accomplish this by splitting a *single* data set into two (i.e., odd-numbered spectra 1−9 and even-numbered spectra 2−10 for a 10 spectra data set). Because the relationship between the signal from the individual components with their respective T_1 is exponential, the requirement of proportionality is satisfied. *Only* the correct solution will show the proportionality. The method is computationally fast and arrives at a solution directly.

For the data analysis MATLAB software (The MathWorks, Natich, MA) was used. The computer configuration is a 200 MHz Pentium Processor with 64 MB RAM. MATLAB functions used for processing the data are described elsewhere,[4] and the code is given in ref 3b.The spectra were inverted, and the data set was split into two, spectra 1−9 and spectra 2−10. DECRA requires that the number of components be defined prior to analysis. Because the analysis is fast, typically a few seconds for a data set this size, it is convenient to try several permutations of spectral ranges and number of components. For this data set we tried both two and three components.

Discussion of Results

Table 1 summarizes the solution and solid-state ^{13}C chemical shifts for **1** together with tentative assignments. The chemical shifts are from the reference spectra shown in Figure 2d,f.

Figure 2 shows a portion of the data set as well as the results of DECRA compared with the reference spectra. As can be seen in Figure 2a,b, changes are observed in the spectrum as a function of the τ value. Some of the peaks decay (or have completely disappeared) with respect to the others. DECRA analysis using two components provides two very different spectra. They are shown in Figure 2c,e. Reference spectra of

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Table 1. ^{13}C Chemical Shift Assignments in Solution and in the Solid State

carbon no.	solution chemical shift	type I chemical shift	type III chemical shift
1	13.4	14.3	12.8
2	20.6	20.6	22.8
3	25.1	25.2	25.1
4	51.2	53.5	51.9
5	148.6 (q)[a]	149.2 (q)	147.9 (q)
6	118.3	124.8	121.7
7	118.3	109.3	109.3
8	130.6	151.0	148.8
9	130.6	114.3	117.1
10	142.5/142.9 (q)	141.6 (q)	142.4 (q)
11	161.8 (q)	162.7 (q)	157.3 (q)
12	96.3 (q)	92.9 (q)	95.9 (q)
13	112.7 (q)	112.9 (q)	112.1 (q)
14	164.1 (q)	166.3 (q)	165.5 (q)
15	121.3 (q)	120.0 (q)	123.4 (q)
16	114.0	109.3	109.3
17	142.5/142.9 (q)	141.6 (q)	142.4 (q)
18	120.2	124.8	121.7
19	107.9	114.3	117.1
20	148.1	151.0	148.8

[a] (q) signifies a quaternary carbon assigned by APT in solution or interrupted decoupling in the solid state.
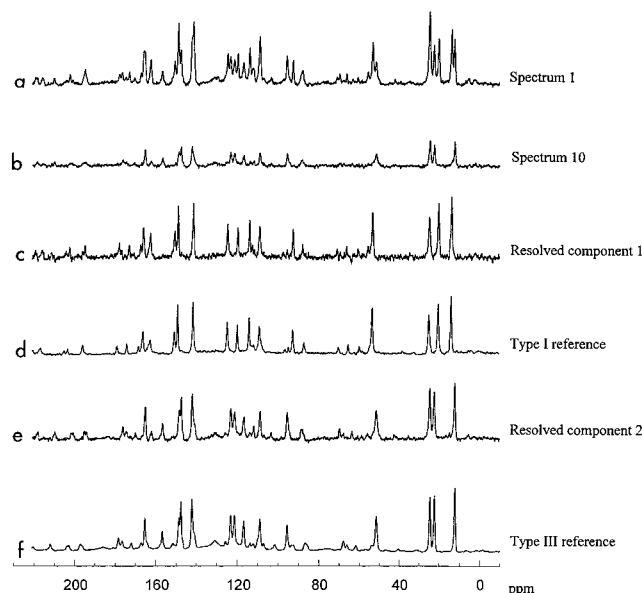
Figure 2. Solid-state ^{13}C CP/MAS spectra of **1** in its two polymorphic forms, type I and type III, and their 1:1 mixture. (a) Spectrum 1 of the data set showing the spectrum of the mixture for an initial τ value of 0.2 s. (b) Spectrum 10 of the data set showing the disappearance of the short T_1 component after a τ interval of 3.8 s. (c) Spectrum of component 1 as resolved from the mixture spectrum by the DECRA method. (d) Spectrum of a pure sample of type I demonstrating its high-fidelity correspondence to component 1. (e) Spectrum of component 2 as resolved by DECRA. (f) Spectrum of a pure sample of type III demonstrating its high-fidelity correspondence to component 2.

pure type I and type III are shown for comparison in Figure 2d,f. Analysis using three components provides two spectra that are identical to the those of the two-component analysis as well as a third spectrum comprised of only noise. This indicates that there are only two components present (based on T_1). The correspondence between the resolved spectra and the reference spectra is excellent.

The resolved T_1 values for type I and type III forms are 1.16 and 7.18 s, respectively, for the two-component analysis and 1.27 and 6.35 s, respectively, for the three-component analysis.

The exponential decay profiles (or concentration profiles) appear slightly "cleaner" for the three-component analysis than for the two-component analysis. We believe that slight baseline artifacts are removed in choosing a third component, which results in more accurate exponentials. The proton T_1 values of the individual pure components were not measured separately, but as a check we determined T_1 values directly from two well-resolved resonances in the data set. For resonances at 13.7 and 12.3 ppm we obtain T_1 values of 1.36 and 6.13 s, respectively.

Conclusions

The work reported here is part of our effort to put in place a set of techniques for NMR-assisted crystallography. We have shown that proton T_1's may function as phase-specific labels for the characterization of mixtures of the various polymorphic forms of an organic solid. We have used compound **1** to illustrate how a chemometric technique, DECRA, can be used in conjunction with solid-state NMR to resolve the complex spectra of such mixtures into the spectra of their constituent phases. We have shown that our curve resolution technique reproduces the reference spectra in high fidelity. In some sense, our technique can be thought of as the solid-state analogue of the diffusion-ordered spectroscopy (DOSY)[9] experiment, accomplishing via proton T_1's and spin diffusion what DOSY accomplishes via molecular self-diffusion.

The generality of this technique remains to be established. At issue is the question of how common measurable differences in proton T_1's are among the different polymorphs of a given compound. Compound **1** as well as some other systems we have examined contains methyl groups, which, when present, usually act as magnetization sinks and thus determine the rate of spin−lattice relaxation in a given organic solid. Differences in packing are known to distort methyl groups sufficiently to cause appreciable changes in the ^{13}C chemical shift tensor.[10] These same distortions may be expected to also modify the dynamics of methyl group rotation. Often, polymorphic behavior can also be induced by differences in crystal hydration, which can lead to changes in proton T_1. Hence, we suggest that "useful" proton T_1 differences among polymorphs may be quite common, especially within a judiciously chosen temperature regime. An additional complication would arise, however, if our technique were applied to systems with microphase separation such as partially crystalline polymers or blends of immiscible polymers. For typical τ values of $\cong 1$ s, spin diffusion between phases with a length scale of less than 30 nm would cause the proton relaxation rates to converge and thus lose their phase-specificity.

As a general method, DECRA, unlike other spectral curve resolution techniques, is relatively insensitive to poor S/N or severe spectral overlap. However, it has low tolerance for experimental artifacts that vary from spectrum to spectrum within a data set because of instrumental instabilities. This may include baseline and phase instabilities or an artifactual departure from exponential behavior due to receiver nonlinearities. These limitations become increasingly critical for closely spaced decay time constants. Conversely, well-separated time constants allow for lower S/N tolerance and greater dynamic range in composition. For example in a PGSE NMR experiment[4c] we were able to resolve five components with diffusivities ranging from 6.06 to 0.14×10^{-9} m^2/s and relative compositions ranging between 5% and 45%. In another experiment[4d] we have shown that a 50-fold difference in the exponential decay rate constant allows

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the resolution of a component at a concentration 50 times less than that of the main component.

In this paper we focused on spectroscopy as a method of mixture analysis in the solid state. We have not discussed the very significant chemical shift differences between the spectra of type I and type III reported in Table 1. Detailed ^{13}C peak assignments and an interpretation of the chemical shift differ-

ences on the basis of differences in conformation and packing will be the subject of a later publication.

While our results were obtained in the context of photographic technology, they have broader significance and should be of particular interest to the pharmaceutical and biomedical industries.